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Director of RWS Group Ltd, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare;

1. That I am a citizen of the United Kingdom of Great Britain and Northern Ireland.

2. That the translator responsible for the attached translation is well acquainted with the French and English languages.

3. That the attached is, to the best of RWS Group Ltd knowledge and belief, a true translation into the English language of the accompanying copy of the specification filed with the application for a patent in France on 15 January 2003 under the number 03/50,002 and the official certificate attached hereto.

4. That I believe that all statements made herein of my own knowledge are true and that all statements made on information and belief are true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application in the United States of America or any patent issuing thereon.

For and on behalf of RWS Group Ltd

The 3rd day of July 2006

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PATENT

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The Director-General of the Institut National de la Propriété Industrielle certifies that the attached document is a true copy of an application for industrial property titleright filed at the Institute.

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On behalf of the Director-General of the
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The Patent Department Head

[signature]

Martine PLANCHE

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Intellectual Property Code – Book VI

REQUEST FOR GRANT

DATE OF SUBMISSION OF THE DOCUMENTS	15/01/2003
NATIONAL REGISTRATION No.	03/50,002
PLACE OF FILING	75
DATE OF FILING	15/01/2003

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L'AIR LIQUIDE
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France

Your file references: S6093 ALSI OP

1 NATURE OF THE APPLICATION			
Patent application			
2 TITLE OF THE INVENTION			
	USE OF XENON OR N2O IN THE TREATMENT OF POST-ISCHAEMIC BRAIN CELL DETERIORATION		
3 PRIORITY DECLARATION OR APPLICATION FOR THE BENEFIT OF THE FILING DATE OF A PRIOR FRENCH APPLICATION	Country or company	Date	No.
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REQUEST FOR GRANT

DATE OF SUBMISSION OF THE DOCUMENTS NATIONAL REGISTRATION No. PLACE OF FILING DATE OF FILING	Marie DUCREUX L'AIR LIQUIDE 75 quai d'Orsay 75321 PARIS CEDEX 07 France
Your file references: XX	

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6 DOCUMENTS AND FILES ATTACHED	Electronic file	Pages	Details
Patent text	textebrevet.pdf	11	D 7, R 3, AB 1
Drawings	dessins.pdf		, figures 8
Designation of the inventors			
7 METHOD OF PAYMENT			
Method of payment	Debit to client account No.		
Client's account No.	516		
8 SEARCH REPORT			
Immediate compilation			

9 FEES ENCLOSED	Currency	Rate	Quantity	Amount to be paid
062 Filing	EURO	0.00	1.00	0.00
063 Search report (S.R.)	EURO	320.00	1.00	320.00
068 Claims from the 11th	EURO	15.00	4.00	60.00
Total to be paid	EURO			380.00

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Signatory: FR, Air Liquide, M. Ducreux
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Function
Accredited representative (First Applicant)

The invention relates to the use of nitrous oxide (N_2O) and/or of xenon or of an N_2O or xenon donor for producing all or part of a medicinal product intended to treat or prevent post-ischaemic brain cell deterioration, in particular deterioration subsequent to a stroke, especially all or part of an inhalable gaseous medicinal product, in humans or animals.

In cerebral ischaemia subsequent to a stroke, and in strokes in general, a functional alteration of many neurotransmission systems is usually noted from a neurochemical point of view, in particular an increase in the release of glutamate, the excitotoxicity and contribution of which to neuronal death are known, as recalled by *Dirnagl et al., Trends Neurosci, 22: 391, 1999*.

Moreover, from a functional point of view, in the case of global ischaemia in the rat, an increase is observed in locomotor activity, in particular described by *Wang and Corbett, Brain Res., 533: 78, 1990; Baldwin et al., Neurodegeneration 2: 139, 1993*, the development of which is generally attributed to an alteration in cognitive functions of spatial recognition rather than to an alteration in sensory-motor functions.

As a result, a potential therapeutic role for ionotropic and metabotropic glutamergic receptor antagonists have been suspected, in particular by *Chazot, Curr Opin Invest Drugs 1: 370, 2000; Drian et al., Neurochem Int 38: 509, 2001*.

It also appears that the deleterious effects of known cerebral ischaemias appear to involve localized ischaemias which are thought to be caused by glutamergic excitotoxicity.

In fact, the therapeutic potential of glutamergic receptor antagonists is often put forward in the treatment of neuropathologies of excitotoxic origin, in particular cerebral ischaemia, as described by *Dirnagl et al., Trends Neurosci 22: 391, 1999*, and productive disorders, as described by *Benes, Brain Res. Review 31: 251, 2000*.

However, the physiology of glutamergic receptors is complex and it appears that the high affinity antagonists may also exhibit neurotoxic properties, according to *Burns et al., Psychopharmacology 115: 516, 1994*.

Thus, a potential therapeutic advantage of low affinity antagonists, in particular for NMDA, has recently been proposed by *Palmer and Widzowski, Amino acids 19: 151, 2000*.

To date, no medical product however exists for preventing or treating, at least partially, post-ischaemic brain cell degradation subsequent to strokes.

The present invention falls within this context, and aims to provide all or part of a medicinal product which can be used for preventing, decreasing or treating any post-ischaemic brain cell deterioration, in particular subsequent to a stroke, in humans or animals.

The invention therefore relates to the use of nitrous oxide (N_2O) and/or of xenon or of an N_2O or xenon donor for producing all or part of a medicinal product intended to treat, minimize or prevent post-ischaemic brain cell deterioration.

Depending on the case, the use of the invention may comprise one or more of the following technical characteristics:

- all or part of the gaseous medicinal product is in inhalable form;

- the post-ischaemic brain deterioration results in or is subsequent to a stroke;

- the xenon or the xenon donor is in gaseous form or is included in a gas or a mixture of gases;

- the nitrous oxide (N_2O) or the nitrous oxide donor is in gaseous form or is included in a gas or a mixture of gases;

- the medicinal product contains an effective proportion of nitrous oxide (N_2O) and/or of xenon or of an N_2O or xenon donor;

- the medicinal product also contains at least one other gaseous compound chosen from oxygen, nitrogen or argon, preferably nitrogen and oxygen;

- the medicinal product contains an amount which is less than 60% by volume of xenon or of xenon donor, preferably less than or equal to 50% by volume;

- the medicinal product contains an amount ranging up to approximately 80% by volume of N_2O or of N_2O donor, preferably up to 75% of N_2O;

- the medicinal product contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

The invention therefore also relates to an inhalable medicinal product with neuroprotective action in the

brain, containing an effective amount of nitrous oxide (N_2O) and/or of xenon or of a donor of such a compound, in particular intended to treat, minimize or prevent post-ischaemic brain cell deterioration.

According to the case, the medicinal product of the invention may comprise one or more of the following technical characteristics:

- it contains an amount ranging up to 80% by volume of gaseous N_2O or an amount which is less than 60% by volume of xenon;

- it also contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

The idea on which the present invention is based is to take advantage of the NMDA receptor antagonist properties of xenon or N_2O for their neuroprotective nature, in prevention or treatment of post-ischaemic pathologies subsequent to strokes.

In fact, recent studies, carried out in vitro, have shown that xenon and N_2O can potentially behave like low-affinity antagonists of glutamergic receptors for N-methyl-D-aspartate, NMDA (*Franks et al., Nature 396: 324, 1998; Jevtovic-Todorovic et al., Nature Med. 4: 460, 199; Yamakura and Harris, Anesthesiology, 20008).*

Based on these observations, experiments were carried out in the context of the present invention, with the aim of determining the neuroprotective effects of N_2O and of xenon, on neuronal death induced by transient cerebral ischaemia in rats.

In order to demonstrate the beneficial effect of administering N_2O or xenon on brain cells subsequent to cerebral ischaemia, adult Sprague-Dawley rats weighing

350 g were subjected to the following experimental protocol.

On day 1, focal ischaemia was induced in each of the rats by middle cerebral artery occlusion (MCAO), for a period of 1 h 30 minutes.

The transient focal cerebral ischaemia by MCAO is obtained conventionally by introducing a flexible nylon thread 1, represented diagrammatically in Figure 1 (length 6.5 mm, diameter 180 μm), a portion 2 of the proximal end of which has a diameter greater than that of the thread (length 3 mm, diameter 380 μm), into the vascular system of the rat, as far as the region of the ipsilateral hemisphere so as to cause an embolism therein, i.e. an ischaemia.

Next, the rats are reperfused for 10 to 20 minutes, and are then made to inhale several mixtures of gases, namely:

- mixture No. 1: air (control)
- mixture No. 2: N_2O (75% vol), the remainder being oxygen (25%)
- mixture No. 3: xenon (50% vol), the remainder being oxygen (20 to 25%) and nitrogen (30 to 25%), respectively
- mixture No. 4: xenon (75% vol), the remainder being oxygen (25%).

On day 2, i.e. 24 hours after reperfusion, the rats are killed, the brains are recovered and frozen, and thin sections 40 μm thick are cut and then stained with cresyl violet, as shown in Figure 5.

The volume of neuronal death is calculated, from the sections obtained after staining, in a conventional

manner using an appropriate, commercially available conventional program.

In fact, as shown diagrammatically in Figure 2, the cerebral ischaemia engenders, in general, in 24 hours, an infarction in the region which has been subjected to ischaemia (penumbra), leading to neuronal death in the brain cells present in a considerable portion of this region.

The results obtained during these measurements have been recorded in Figures 3a to 3d, which make it possible to visualize the post-cerebral ischaemia neuroprotective effect of mixtures No. 2 to 4 above, in comparison with mixture No. 1 (air) which serves as a control.

Thus, Figure 3a clearly shows that inhalation by the rats of xenon (Xe) or of nitrous oxide (N_2O) subsequent to an ischaemia makes it possible to considerably reduce the total volume of infarction, since a decrease in this volume of approximately 50% can be achieved in the case of inhalation of mixtures No. 2 and No. 3 instead of air (mixture No. 1 acting as control), and of approximately 30% when mixture No. 4 is inhaled. In this respect, it will also be noted that inhalation of 50% by volume of xenon (mixture No. 3) is more effective than inhalation of a higher dose of xenon, namely 75% (mixture No. 4), which implies that the most effective dose appears to be closer to 50% than to 75% with regard to xenon.

Figures 3b to 3d confirm the results of Figure 3a, since they make it possible to observe that inhalation of xenon or of N_2O makes it possible to decrease, respectively, the post-ischaemic volume of cortical infarction (Fig. 3b), the post-ischaemic volume of striatal infarction (Fig. 3c) and the post-ischaemic

volume of oedema (Fig. 3d), compared to inhalation of air (control = mixture No. 1).

Based on this observation, complementary examinations were carried out in order to determine the neurotoxic effects of the xenon and of the nitrous oxide (N_2O), at various amounts, compared to air, on brain receptors of the NMDA type.

The results of these examinations are reported in Figure 4, which clearly shows that the administration of xenon or of nitrous oxide engenders a smaller volume (in mn^3) of deteriorated NMDA receptors than the control (air), this being with the nitrous oxide given at a dose of 50% or 75% by volume (remainder = 25% of O_2) and the xenon given at a dose of 50% or 75% (remainder = mixture of 25% of O_2 + 25% of N_2, or, respectively, 25% of O_2).

However, a neurotoxic effect which is variable according to the dose administered thus emerges, leading to the observations that N_2O at 75% and xenon at 50% by volume are more neuroprotective than N_2O at a dose of 50% and xenon at a dose of 75%.

In other words, these data confirm that administration by inhalation of xenon at a dose of 50% by volume (or less) or of N_2O at a dose of 75% by volume (or less) engenders a neuroprotective action with respect to cerebral ischaemia and other similar excitotoxic diseases.

The inhalable medicinal product according to the invention is packaged in pressurized gas containers, such as gas bottles, and is dispensed to the patient via an appropriate system for administering gas, equipped with a breathing mask, a tracheal catheter, or the like.

Claims

1. Use of nitrous oxide (N_2O) and/or of xenon or of an N_2O or xenon donor, for producing all or part of a medicinal product intended to treat, minimize or prevent post-ischaemic brain cell deterioration.

2. Use according to Claim 1, characterized in that all or part of the gaseous medicinal product is in inhalable form.

3. Use according to either of Claims 1 and 2, characterized in that the post-ischaemic brain deterioration results in or is subsequent to a stroke.

4. Use according to one of Claims 1 to 3, characterized in that the xenon or the xenon donor is in gaseous form or is included in a gas or mixture of gases.

5. Use according to one of Claims 1 to 4, characterized in that the nitrous oxide (N_2O) or the nitrous oxide donor is in gaseous form or is included in a gas or in a mixture of gases.

6. Use according to one of Claims 1 to 5, characterized in that the medicinal product contains an effective proportion of nitrous oxide (N_2O) and/or of xenon or of an N_2O or xenon donor.

7. Use according to one of Claims 1 to 6, characterized in that the medicinal product also contains at least one other gaseous compound chosen from oxygen, nitrogen or argon, preferably nitrogen and oxygen.

8. Use according to one of Claims 1 to 7, characterized in that the medicinal product contains an

amount which is less than 60% by volume of xenon or of xenon donor, preferably less than or equal to 50% by volume.

9. Use according to one of Claims 1 to 8, characterized in that the medicinal product contains an amount ranging up to 80% by volume of N_2O or of N_2O donor, preferably up to 75% of N_2O.

10. Use according to one of Claims 1 to 9, characterized in that the medicinal product contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

11. Inhalable medicinal product with neuroprotective action in the brain, containing an effective amount of nitrous oxide (N_2O) and/or of xenon or of a donor of such a compound.

12. Medicinal product according to Claim 11, characterized in that it contains an amount ranging up to 80% by volume of N_2O. or an amount which is less than 60% by volume of xenon.

13. Medicinal product according to either of Claims 11 and 12, characterized in that it also contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

14. Pressurized gas container containing a medicinal product according to one of Claims 11 to 13, in particular a gas bottle.

Claims

1. Use of nitrous oxide (N_2O) or of an N_2O donor, for producing all or part of a medicinal product intended to treat, minimize or prevent post-ischaemic brain cell deterioration.

2. Use according to Claim 1, characterized in that all or part of the gaseous medicinal product is in inhalable form.

3. Use according to either of Claims 1 and 2, characterized in that the post-ischaemic brain deterioration results in or is subsequent to a stroke.

4. Use according to one of Claims 1 to 3, characterized in that the medicinal product also contains xenon or a xenon donor, the xenon or the xenon donor being in gaseous form or being included in a gas or mixture of gases.

5. Use according to one of Claims 1 to 4, characterized in that the nitrous oxide (N_2O) or the nitrous oxide donor is in gaseous form or is included in a gas or in a mixture of gases.

6. Use according to one of Claims 1 to 5, characterized in that the medicinal product contains an effective proportion of nitrous oxide (N_2O) and/or of xenon or of an N_2O or xenon donor.

7. Use according to one of Claims 1 to 6, characterized in that the medicinal product also contains at least one other gaseous compound chosen from oxygen, nitrogen or argon, preferably nitrogen and oxygen.

8. Use according to one of Claims 1 to 7, characterized in that the medicinal product contains an amount which is less than 60% by volume of xenon or of xenon donor, preferably less than or equal to 50% by volume.

9. Use according to one of Claims 1 to 8, characterized in that the medicinal product contains an amount ranging up to 80% by volume of N_2O or of N_2O donor, preferably up to 75% of N_2O.

10. Use according to one of Claims 1 to 9, characterized in that the medicinal product contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

11. Inhalable medicinal product with neuroprotective action in the brain, containing an effective amount of nitrous oxide (N_2O) or of a donor of such a compound.

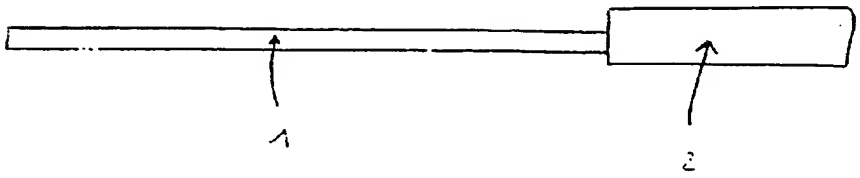
12. Medicinal product according to Claim 11, characterized in that it contains an amount ranging up to 80% by volume of N_2O.

13. Medicinal product according to Claim 11, characterized in that it also contains xenon or a donor of such a compound, preferably in an amount which is less than 60% by volume of xenon.

14. Medicinal product according to one of Claims 11 to 13, characterized in that it also contains from 19 to 25% by volume of oxygen and, optionally, of nitrogen.

15. Pressurized gas container containing a medicinal product according to one of Claims 11 to 14, in particular a gas bottle.

FIG 1



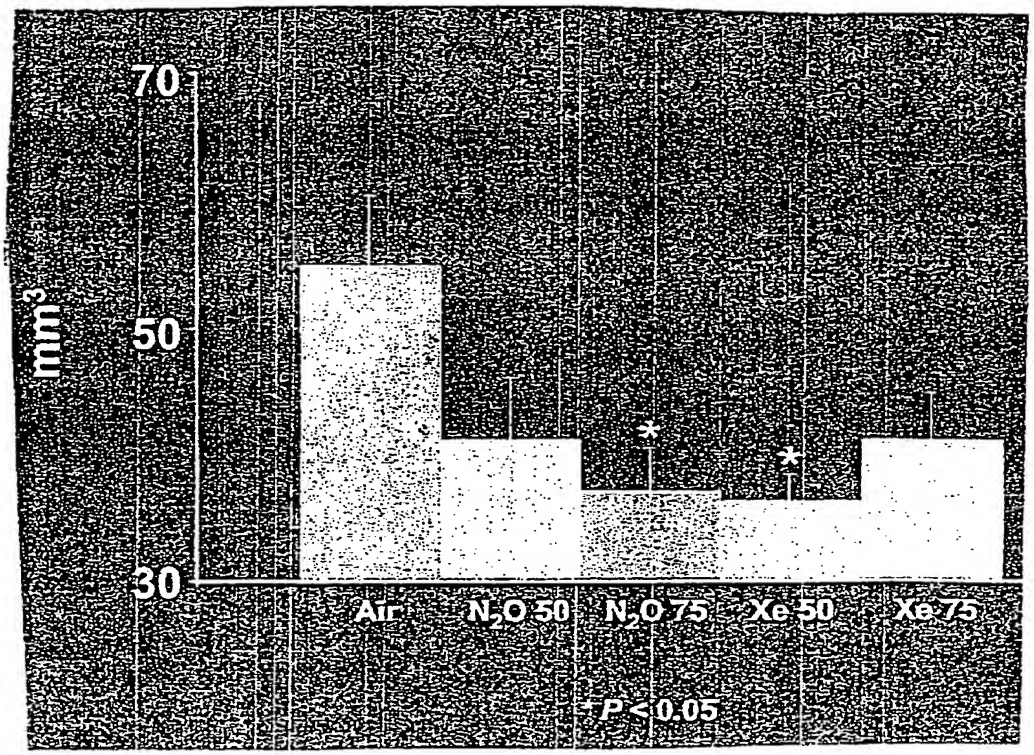


FIG 4

received on 27/06/03

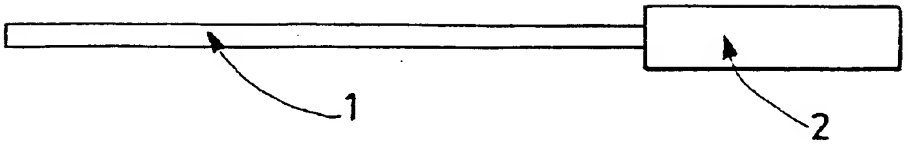


FIG.1

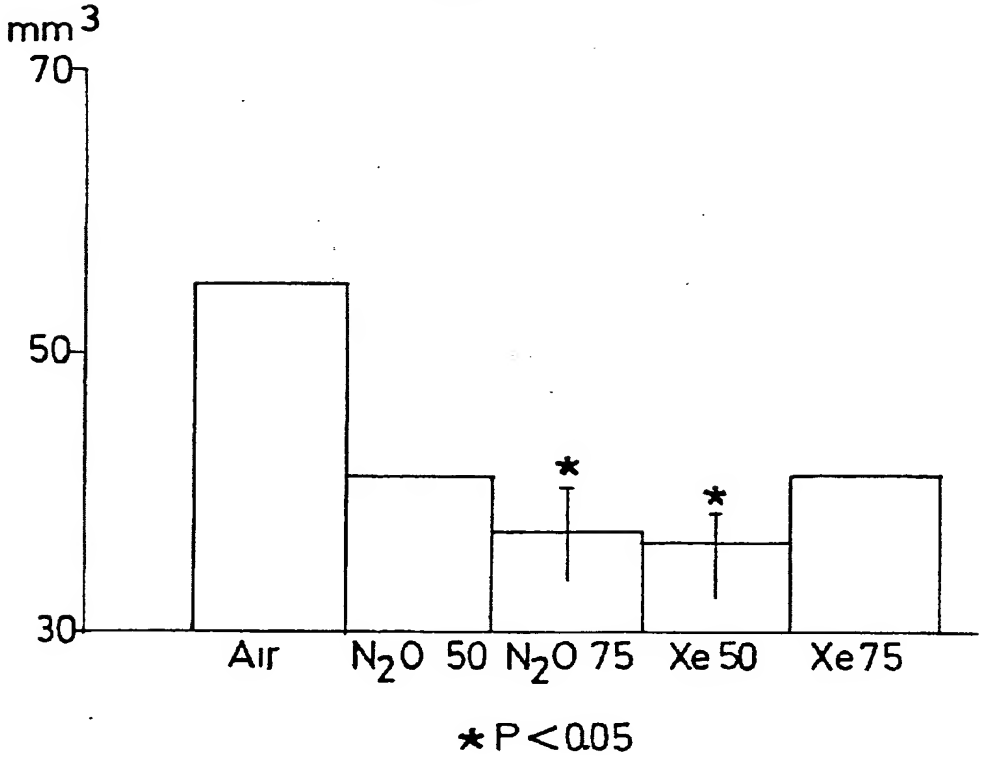


FIG.4

Fig. 1

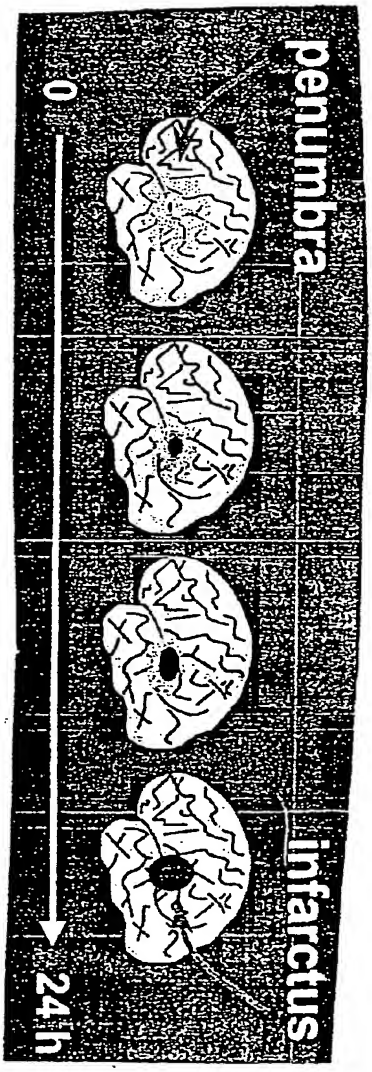


Fig. 2

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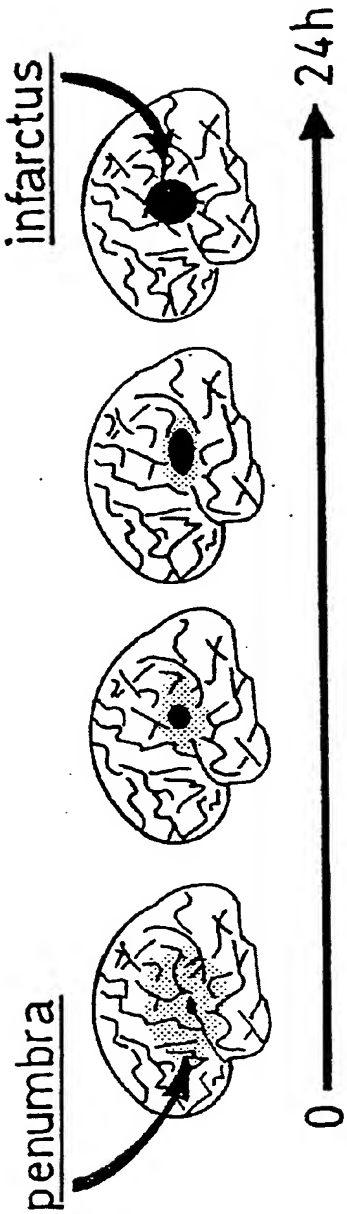

FIG.2

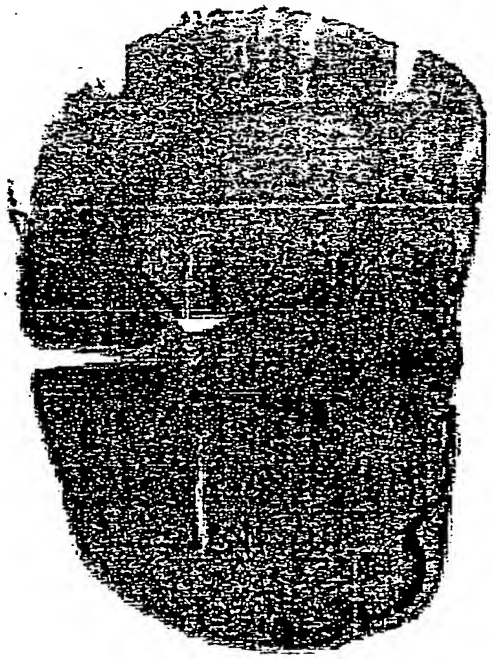

FIG.5

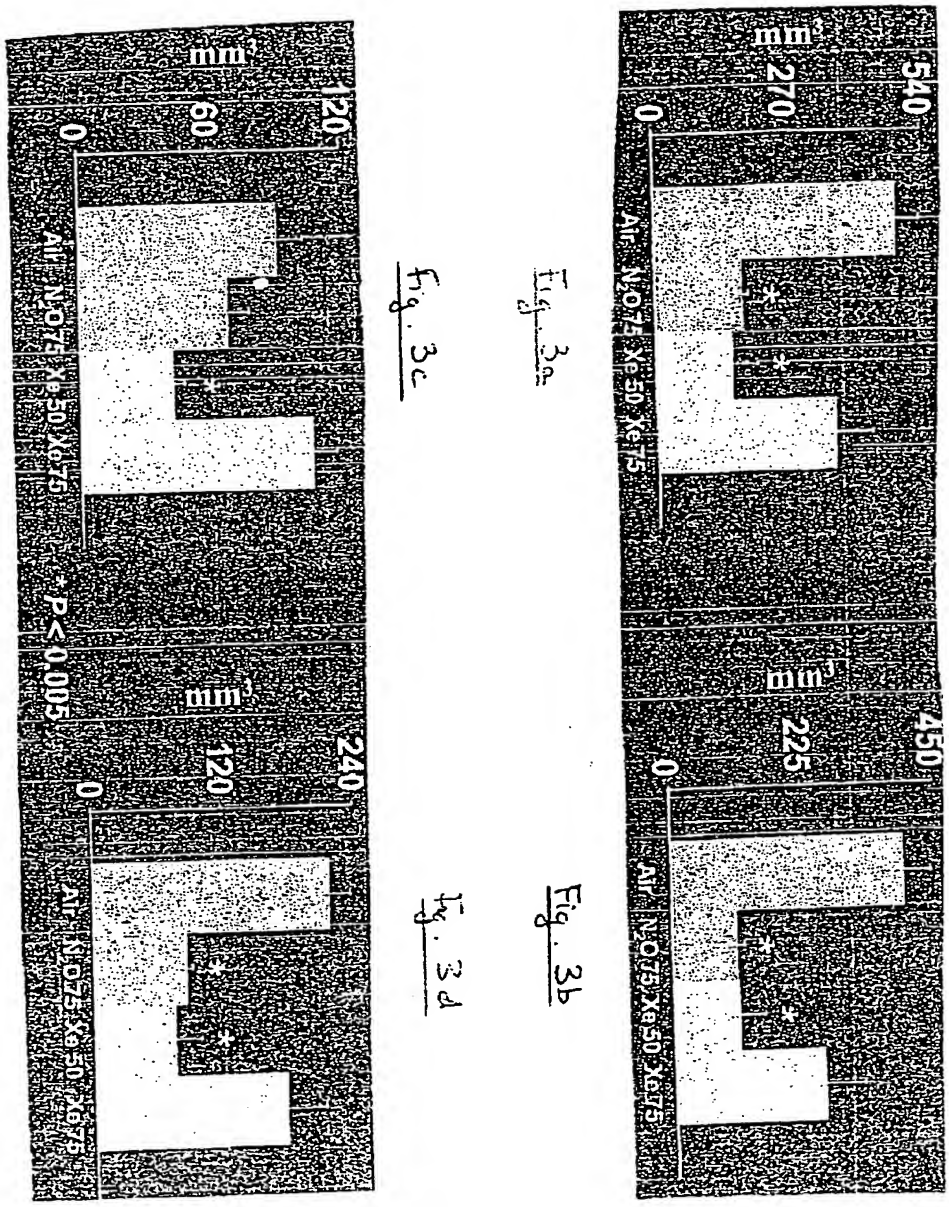

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3d

received on 27/06/03

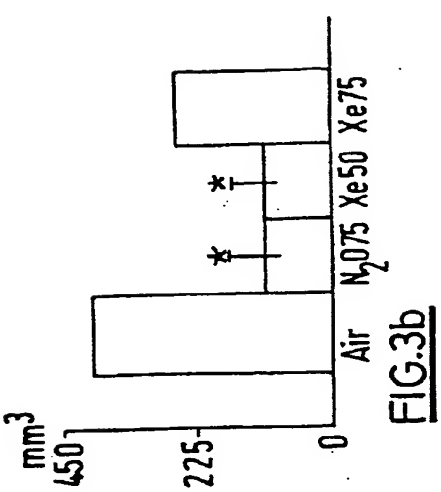

FIG.3b

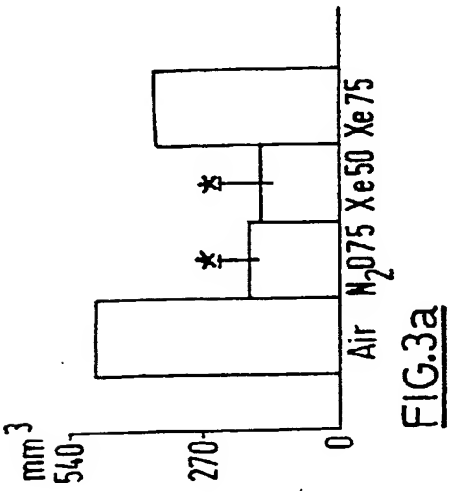

FIG.3a

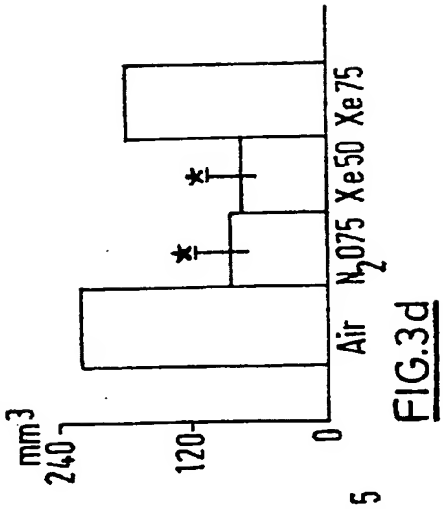

FIG.3d

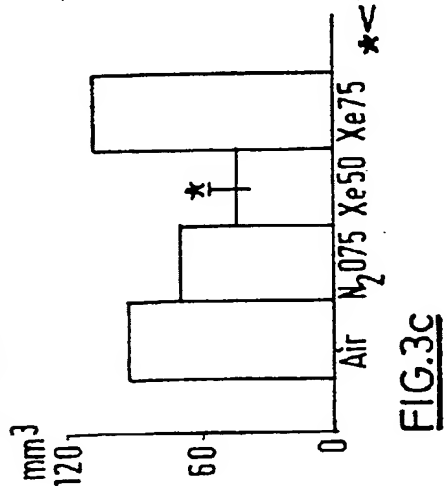

FIG.3c

received on 24/01/03

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PATENT
CERTIFICATE OF UTILITY

DESIGNATION OF THE INVENTOR

Your file references	S6093 ALSI OP
National Registration No.	
TITLE OF THE INVENTION	USE OF XENON OR N2O IN THE TREATMENT OF POST-ISCHAEMIC BRAIN CELL DETERIORATION
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DESIGNATE(S) AS INVENTOR(S):	
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Employer company	

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